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STUDIES ON THE MICROSCOPIC SLIDE-AGGLUTINATION TEST FOR Q FEVER *

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SYNOPSIS

The author has devised a micro-agglutination test for the serodiagnosis of Q fever. Preliminary studies had already indicated that it was highly sensitive, and this has now been confirmed. Using the sera of sheep infected with Q fever, the microscopic slide-agglutination test was found to be approximately 10 times as sensitive as the capillary agglutination test and the macroscopic tube-agglutination test, and more than 20 times as sensitive as the complement-fixation test. The sensitivity of the test can be still further enhanced by the addition of inactivated bovine serum ("auxi-agglutination") or by the addition of bovine serum and complement ("conglutination"), but for routine diagnosis or epidemiological studies, the test is sufficiently sensitive without introducing these complications.

In 1952, Babudieri & Secchi described a new microscopic agglutination technique for the serodiagnosis of Q fever. A subsequent note (Babudieri, 1954) compared the sensitivity of this method for the detection of specific antibody with that of the capillary agglutination test (Luoto, 1953) and of the complement-fixation test. In the present communication a more extensive comparison is made and some of the factors underlying the microscopic agglutination reaction are discussed.

Material and Methods

Preparation of the antigen

The " Grottazzolina " strain of *Coxiella burnetii*, isolated in 1949 from a patient in Italy, is used. This strain is maintained by yolk-sac passage in hens' eggs of 7 days' embryonation.

To prepare the antigen, 0.5 ml of a 1% suspension of infected yolk sac is inoculated into the yolk sac of 7-day old chick embryos. Five to 7 days after inoculation, when deaths begin to occur among the inoculated embryos,

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the yolk sacs of the surviving eggs are harvested. The yolk sacs are cultured for bacteriological sterility and smears made to determine whether the rickettsiae are present in large numbers.

The infected yolk sacs are placed in a Waring blendor and physiological saline solution containing 3% of formalin is added in the proportion of 10 ml per yolk sac. The yolk sacs are minced in the blendor for 3 minutes, after which the jar is placed in the refrigerator at 4°C and kept at this temperature for 4 days. After this interval, the suspension is centrifuged for one hour at 10000 r.p.m., the supernatant fluid is removed, and the sediment is re-suspensed in physiological salt solution, using 2 ml per yolk sac. To this suspension an equal volume of ethyl ether is added and the mixture is poured into a separating funnel and placed for 24 hours in the refrigerator. The aqueous phase is then removed, and an equal volume of physiological salt solution is added to the ether phase, with continuous stirring. This mixture is kept for 24 hours in the refrigerator, after which the aqueous phase is separated and added to that removed on the preceding day, while the ether phase is discarded.

The pooled aqueous phases are centrifuged for 20 minutes at 1000 r.p.m. and the supernatant fluid is removed and saved. The sediment is re-suspensed in physiological salt solution (1 ml per yolk sac) and centrifuged at the same speed. The resulting supernatant fluid is added to the previous one, and the sediment discarded. The pooled supernatant fluids are centrifuged for one hour at 10 000 r.p.m., the supernatant fluid is removed, and the sediment is resuspended in physiological salt solution. The whole cycle of high- and low-speed centrifugations is repeated two to four times until microscopic examination shows that a pure suspension of rickettsiae has been obtained. (If necessary, the extraction with ethyl ether is repeated.) The final step in the preparation of the antigen is exposure of the pure rickettsial suspension to ultrasonic vibration (frequency 1 megacycle per sec., intensity 8 watts per cm^2 for 10 minutes). This treatment gives excellent dispersion of the rickettsiae. A final microscopic check is made to be sure that the suspension is completely homogeneous and is free from clumps of rickettsiae. Merthiolate is added to the antigen in a final concentration of 1: 10 000.

The antigen is titrated by testing it in serial dilutions against serial dilutions of a negative serum and against a known positive serum diluted to its endpoint agglutinating titre. The antigen should give no evidence of reactivity with the negative serum. The highest dilution of antigen giving a clear-cut agglutination with the positive serum diluted to its endpoint titre is the antigen dilution to be used for the detection and assay of Q fever agglutinins in serum. In practice, the extent to which the antigen is diluted for titration purposes can be qualitatively ascertained by comparing the opaqueness of the unknown antigen with that of a standardized antigen. The titre of the dilution varies from batch to batch, depending upon the

rickettsial content of the yolk sacs that are used ; in general, the stock antigen can be diluted from 1:100 to 1:300 for use in the tests. The stock antigen should be stored in a refrigerator (freezing is to be avoided) and diluted just before use.

The microscopic agglutination test

The test is performed on special glass slides. Any design which prevents admixture of a series of antigen mixtures and which permits microscopic examination of the mixtures is satisfactory. The slides used by us are made of double-strength window glass cut to 3¼ in. × 6 in. (8.25 cm × 15.25 cm) and have bevelled edges. The surface is etched, except for a series of circles 3/8 in. (9.5 mm) in diameter, arranged in 10 rows of 5 circles each. The circles should be separated by at least 1/8 in. (3 mm), and the area within each circle must be perfectly smooth. The etching serves to prevent liquid deposited within the circles from overflowing and mixing with liquid from adjacent circles. When such slides are not available commercially, satisfactory substitutes can be prepared in the laboratory. A rubber stopper of the appropriate diameter is dipped in melted paraffin wax and used to mark out the circular areas on the glass slide. The slide is then exposed to the vapour of hydrofluoric acid, which etches the area of glass not protected by the wax.

The serum to be examined is inactivated at 56°C for 30 minutes. If the specimen is cloudy or contaminated, it is centrifuged prior to use. Each serum is diluted serially in twofold steps beginning with 1:4 and ending with 1:64. Dilutions are prepared in small tubes, using plain or buffered physiological saline solution containing 1 part in 10 000 of merthiolate. By means of a 0.1 ml pipette graduated in hundredths, 0.02 ml of the diluted antigen is placed in each of the 50 circular areas on one of the special slides. To each area an equal volume of serum dilution under test is then added. The same pipette may be used for each serum, care being taken to begin which the highest dilution and proceed to the lowest. A platinum needle, sterilized by flaming, is used to mix the serum and antigen and to spread the mixture over the surface of the entire circular area or cell. Each series of tests contains 3 controls : one control consists of antigen and dilutions of known positive serum, another of antigen and dilutions of known negative serum, and the third of a simple mixture of antigen and physiological saline solution with merthiolate. After all the serum-antigen mixtures have been prepared, the slide is placed in a moist chamber (a large Petri dish containing a wad of cotton wool soaked in water will suffice) and left at room temperature overnight.

On the following day, the slide is removed from the moist chamber and placed in an incubator at 37°C to dry. The drying should be done slowly and should take at least 30 minutes. When dry, the slide is immersed in

methyl alcohol for 3 minutes. The alcohol is then poured off and, without washing, the slide is covered with Giemsa stain and left for 20 minutes. At the end of this time, it is washed under tap-water, left to dry, and then examined under the microscope. In general, examination with a high, dry objective in a magnification of about × 100-150 is satisfactory. The preparation can frequently be clarified by smearing it with a little cedar oil. When difficulty is experienced in making readings with a dry objective, an oil-immersion objective may be required.

Agglutination is indicated by the presence in the centre of the circular area of largish, compact, violet-coloured agglomerations of rickettsiae. Large agglomerations of rickettsiae are generally found in the first dilutions of positive sera ; the clumps become smaller and scarcer in successive dilutions until, at the endpoint of the serum, only a very few clumps containing small numbers of rickettsiae are seen. If agglutination occurs even with the highest dilution of a serum, the test is repeated until the agglutinating endpoint titre of the serum is reached. If the serum is negative, not only are there none of the large clumps described above, but even single stained rickettsiae are virtually absent or encountered only in very small numbers.

In some instances, crystals of sodium chloride as well as stained particles from the serum may interfere with the reading of the results in the initial serum dilution (1:4). No trouble is generally encountered, however, with the second dilution in the series (1:8).

Results

Previous studies (Babudieri & Secchi, 1952 ; Babudieri, 1954) on more than 700 serum specimens obtained from experimentally infected guinea pigs and sheep, from humans with Q fever and other infectious diseases, and from healthy humans and animals, have established the specificity of the microscopic slide agglutination test. These investigations also showed that the results of the agglutination test do not always agree with those of the complement-fixation test. In general, patients' serum does not give a positive agglutination test until two or three days after the complement-fixation test has become positive. However, agglutinating antibody tends to persist for a much longer period than does complement-fixing antibody, and may be present in the serum of an individual months and even years after his recovery from the illness.

Sensivity of the test

In previous studies (Babudieri, 1954), the sensitivity of the microscopic agglutination test was compared with that of the complement-fixation test and of the capillary agglutination test (Luoto, 1953). In general, it was

found that in patients suffering from an infectious disease, or recently recovered from an infection, the sensitivity of the agglutination test was only slightly less than that of the complement-fixation test but appreciably higher than that of the capillary agglutination test.

It seemed desirable, however, to extend these comparative studies, and for this purpose the sera of 72 sheep were utilized. These animals were part of a larger flock which had been kept under continuous observation for several years by Dr. Lennette and his associates in California. The 72 sheep under consideration were serologically negative animals which had been imported from an area where Q fever is not endemic. After arrival in California, the animals were placed in contact with an infected flock, and the evolution of Q fever infection in these immigrant animals was studied over a three-year period by Lennette et al. The animals had been bled at frequent intervals, and serum specimens were available for comparative studies.

The sera were examined by the following procedures : (a) Luoto's capillary agglutination method, (b) macroscopic agglutination in tubes, using the Henzerling antigen and the technique previously described by Lennette et al. (1952), (c) the microscopic slide-agglutination test as described on p. 983, and (d) the complement-fixation test using a Henzerling strain antigen and the technique described by Lennette, Clark & Dean

TABLE 1. COMPARATIVE SENSITIVITIES OF THE MICROSCOPIC SLIDE-AGGLUTINATION TEST AHD THE COMPLEMENT FIXATION TEST FOR DEMONSTRATING ANTIBODY TO *COXIELLA BURNETTI*

Microscopic slide test		Number of sera with complement titres of					
Titre	Number of sera *	<1 : 2	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32
<1 : 4	8	6	1	0	1	0	0
1 : 4	2	2	0	0	0	0	0
1 : 8	0	0	0	0	0	0	0
1 : 16	3	3	0	0	0	0	0
1 : 32	7	6	1	0	0	0	0
1 : 64	22	11	3	6	2	0	0
1 : 128	15	4	0	4	3	3	1
1 : 256	8	3	0	1	1	1	2
1 : 512	3	1	0	0	0	1	1
1 : 1024	1	0	0	0	0	0	1
Totals	69 *	36	5	11	7	5	5

* Three sera were omitted from this table because they were anticomplementary.

(1949). Results of the comparative tests are shown in Tables 1 and 2, in which the test sera are grouped according to the titres obtained in each type of test. Table 1 deals with only 69 of the 72 sera, since three specimens were anticomplementary. It will be noted that the sera which failed to fix complement, or did so only in very low dilutions (1: 2 or 1:4), tended to give a positive reaction in the microscopic agglutination test. Thus of 36 sera which showed no demonstrable capacity to fix complement (<1:2), all but 6 were positive (1:4 or greater) in the microscopic agglutination test. Similarly, of 5 sera which fixed complement in a 1:2 dilution, 4 were positive by the agglutination test and in comparatively high titre. A useful index of the difference in sensitivity between the two tests is obtained by multiplying the number of sera which reacted to each titre by the value of the titre and adding the products. When the data in Table 1 are treated in this way, a value of 350 is obtained for the complement-fixation test and a value of 8216 for the microscopic slide-agglutination test. On this basis, the microscopic slide-agglutination test is approximately 23 times more sensitive than the complement-fixation method that was used.

In Table 2 the results obtained in the microscopic agglutination test are compared with those obtained in the capillary agglutination test and in the tube or macroscopic agglutination test. It will be noted that the microscopic method was somewhat more sensitive than the other two,

TABLE 2. COMPARATIVE SENSITIVITIES OF THE MICROSCOPIC SLIDE TEST, THE MACROSCOPIC TUBE TEST, AND THE CAPILLARY TUBE TEST FOR DEMONSTRATING ANTIBODY TO *COXIELLA BURNETTI*

Microscopic slide test		Macroscopic tube tests: number of sera with titres of							Capillary tube test: number of sera with titres of						
Titre	Number of sera	<1:2	1:2	1:4	1:8	1:16	1:32	1:64	<1:2	1:2	1:4	1:8	1:16	1:32	1:64
<1:4	9	7	1	0	1	0	0	0	8	1	0	0	0	0	0
1:4	2	2	0	0	0	0	0	0	2	0	0	0	0	0	2
1:8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:16	3	3	0	0	0	0	0	0	2	0	1	0	0	0	0
1:32	8	7	1	0	0	0	0	0	3	3	0	2	0	0	0
1:64	22	5	7	4	4	1	0	1	2	10	5	2	2	0	1
1:128	15	0	2	3	3	4	2	1	0	1	1	6	6	1	0
1:256	8	0	0	2	1	2	2	1	0	0	2	2	2	0	2
1:512	4	1	0	0	0	0	1	2	0	0	0	0	0	1	3
1:1024	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Totals	72	25	11	9	9	7	6	5	17	15	9	12	10	2	7

since it frequently gave a positive result when the other methods did not, so long as the titres did not exceed 1:64. When the titres were higher than this, all three types of agglutination reaction were positive, although in varying degree. The one exception was a serum that gave a positive reaction with a titre of 1:512 in the microscopic agglutination test and with a titre of 1:32 in the capillary agglutination test, but was negative in the tube agglutination test. In only one case did the tube agglutination test give a positive titre (1:8) when the microscopic test gave a negative result. Since this serum was also negative by the capillary method and by the complement fixation test, the specificity of the positive finding is in question.

If the sensitivity of the three agglutination methods is estimated according to the procedure outlined above, the following indices are obtained : (a) microscopic agglutination, 8760 ; (b) capillary agglutination, 834 ; (c) tube agglutination, 754. Since the endpoint titres were not determined for 8 sera in the capillary agglutination test and for 2 in the tube agglutination test, the indices for these two methods are actually somewhat higher. Using the indices computed from the data in Table 2, the microscopic agglutination test appears to be approximately 10 times as sensitive as either of the other two agglutination methods.

Factors inhibiting agglutination

Sera to be tested for complement-fixing antibody to *C. burnetii* are commonly inactivated between 56°C and 60°C ; differences in temperature within this range do not, on the whole, appear to produce marked differences in antibody titre. Earlier observations have indicated that certain human sera retain their ability to agglutinate *C. burnetii* even when inactivated at 60°C or 62°C, whereas others completely lose their agglutinating power when heated to 60°C. I have noted such behaviour with human sera, but not with guinea-pig sera. It might be considered therefore that agglutinins to *C. burnetii* are in some respects thermolabile, whereas complement-fixing antibodies to this organism are more heat resistant. Streng (1909 a) showed that different antibodies (H and O agglutinins) present in the same serum may vary in thermal sensitivity, and that the thermolability of the same antibody may vary according to the animal species.

An inquiry into the thermal sensitivity of the agglutinin to *C. burnetii* has given no evidence that this antibody is unusually thermolabile ; as shown in Table 3, heating human sera to 60°C or 62°C did not noticeably affect the endpoint titre, but the heated sera may give rise to a prozone phenomenon. There is an indication (see serum No. 1) that the higher the temperature, the greater the initial zone of non-reactivity, i.e., the greater the displacement towards the right of the first dilution that gives a positive

TABLE 3. EFFECT OF HEAT ON AGGLUTININS TO *COXIELLA BURNETTI* AS DEMONSTRATED BY THE MICROSCOPIC AGGLUTINATION TEST

Serum number	Inactivation temperature *	Agglutinating activity of serum diluted								
		1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024
1	56°C	++++	++++	++++	++++	++++	+++	+	—	—
	60°C	—	±	+	++++	++++	+++	+	—	—
	62°C	—	—	—	—	+++	+++	+	—	—
2	56°C	—	+++	+++	++++	++++	++++	++	++	±
	62°C	—	+++	+++	++++	++++	++++	++	++	±
3	56°C	++++	+++	+++	++	++	±	—	—	—
	62°C	—	—	—	±	—	—	—	—	—
4	56°C	++++	+++	+++	+++	+++	+++	+	—	—
	62°C	—	—	—	—	±	++	±	—	—
5	56°C	++++	++++	++++	+++	+++	+++	+++	+	±
	62°C	—	—	—	+	+++	+++	+	±	±
6	56°C	++++	++++	+++	++	++	+	—	—	—
	62°C	++++	+++	+++	++	+	—	—	—	—
7	56°C	++++	+++	+++	++	+	—	—	—	—
	60°C	—	—	—	—	—	—	—	—	—

* Inactivation was for 25 minutes at temperature indicated.

The signs —, ±, ++, +++, ++++ indicate, empirically, the degree of agglutination, determined both from the number and the dimensions of the agglomerates of rickettsiae.

reaction. A prozone of limited degree may sometimes occur even with sera inactivated at 56°C (see serum No. 2). Some sera (see serum No. 6) are apparently unaffected by temperatures as high as 62°C, whereas others (e.g. serum No. 7) may completely lose their agglutinating capacity even at a lower temperature.

A possible explanation of these findings may be the presence of a factor, perhaps a normal component denatured by heating, which inhibits agglutination, but which, in higher serum dilutions, is no longer sufficiently concentrated to exercise its inhibitory activity. Shibley (1929) has attributed the prozone phenomenon to a partial transformation of agglutinins into agglutinoids with a greater affinity for the micro-organisms. When present in adequate concentration, these agglutinoids would then be able to block the receptors of the micro-organisms and prevent their coming into contact with the unaffected agglutinins.

TABLE 4. DESTRUCTION BY HEAT OF AGGLUTINATION-INHIBITING SUBSTANCES PRESENT IN FRESH SERUM

Human serum		Agglutinating activity of serum diluted						
Number	Status	1 : 4	1 : 8	1 : 6	1 : 32	1 : 64	1 : 128	1 : 256
1	Fresh	—	±	—	—	—	—	—
	Inactivated*	+++	++++	+++	+++	++	—	—
2	Fresh	—	—	±	+	++	±	—
	Inactivated*	++	+++	+++	+++	++	++	+

* 56° C for 25 minutes

On the other hand, fresh non-inactivated sera are not devoid of factors which inhibit agglutination. The inhibitory activity is manifested either by the appearance of a prozone or by reduction in the endpoint agglutinating titre. Two examples are shown in Table 4, one serum showing a clear inhibitory activity at practically all dilutions when in the fresh state, and the other showing a prozone reaction.

The agglutination-inhibiting factor present in fresh sera has been studied by van Loghem (1908), Streng (1909 b), Nema (1923), Chiari & Loeffler (1924), and most recently by Thjotta & Jonsen (1949). The studies of Babudieri & Zardi (1956) suggest that the factor interfering with the agglutination of *C. burnetti* is identical with complement, or at least closely related to it. For example, agglutination-inhibiting factor disappears with inactivation or aging of the sera, concomitantly with the loss of complementary activity ; inactivation of guinea-pig serum by heat leads to disappearance of the inhibitor (destruction of fractions C_1 and C_2 of the complement) ; and treatment of fresh sera with alkalis such as sodium bicarbonate or ammonium hydroxide (destruction of fractions C_3 and C_4 of complement) likewise destroys the inhibitory capacity. Reconstitution of the complement by restoration of the destroyed fractions leads to reappearance of inhibitory activity.

The presence of the agglutination-inhibiting factor in fresh sera necessitates heat inactivation of the specimen prior to its use in the micro-agglutination test.

Finally, it seems possible that other agglutination-inhibiting factors may exist, different from those mentioned above and still essentially undefined. One such factor might be identical with the factor first described by Coca & Kelley (1921), which produces such extensive zoning as to make the seroreaction (in brucellosis) essentially negative. Similar observations have been reported by Jones & Orcutt (1934).

Factors enhancing agglutination

A study was made of the " conglutination " and the " auxi-agglutination " phenomena and their possible value in enhancing the sensitivity of the micro-agglutination test.

" Conglutination " was the name given by Bordet & Streng (1909) to a phenomenon which was first observed by Ehrlich & Sachs in 1902. According to Hole & Coombs (1947 a, 1947 b), the serum of cattle and other animals contains a protein of high molecular weight called " conglutinin ". Under certain specific conditions, the conglutinin will attach itself to sensitized antigen which has adsorbed complement, the practical advantage being that it enhances agglutination and makes the reaction easier to read. This is especially valuable in cases where the amount of antigen is very small, as in the micro-agglutination test for Q fever ; and in fact, Barber (1955) has successfully used the conglutination reaction for the diagnosis of Q fever. Barber was able to obtain titres from 30-50 times greater than those obtainable by means of simple agglutination techniques.

Dean (1911) has shown that the sera of guinea-pigs and of other animals can reinforce agglutination, even in the absence of complement—a phenomenon that has been called " auxi-agglutination ".

A number of experiments were conducted on the influence of bovine serum on the micro-agglutination test. It was found that bovine serum, even if inactivated, exerts an inhibiting rather than an enhancing effect if used in low dilutions. If sufficiently diluted, however, bovine serum was found to enhance agglutination. To determine the optimal dilutions of bovine serum to employ, the following procedure was used. An inactivated human serum with a micro-agglutination titre of 1:64 was diluted 1:32 (two agglutinating units) with physiological saline solution. Serial twofold dilutions of inactivated bovine serum were prepared in physiological saline and mixed with equal volumes of the diluted human serum. The optimal dilution of bovine serum was considered to be that dilution which gave maximal reinforcement of agglutination. This usually proved to be 1:16, which is practically the same as the dilution (1:20) recommended in conglutination tests by Hole & Coombs (1947 b) and by Barber (1955).

It should be mentioned that bovine sera exert an " auxi-agglutinating " action only when fresh, and have little or no effect when they have been stored for some time. The difference in activity between freshly drawn and stored bovine sera is illustrated in Table 5 (the bovine sera were inactivated for 20 minutes at 56°C and were used in a dilution of 1:20). It is evident that the auxi-agglutinins in bovine serum are chronolabile. In this they resemble the conglutinins, and like the conglutinins they can probably be preserved only if the serum is lyophilized or kept in the frozen state at a temperature of −20°C or less. As shown in Table 5, the use of fresh bovine serum, inactivated and diluted 1:20, as a diluent for the sera being

TABLE 5. AUXI-AGGLUTINATING ACTION OF BOVINE SERA AND CHRONOLABILITY OF THE AUXI-AGGLUTININ

Q-fever-immune human serum		Agglutinating titre of immune serum						
Number	Diluent	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256
1	Saline, 0.85 %	++	++	++	+	—	—	—
	Stored bovine serum	++	+++	+++	++	±	—	—
	Fresh bovine serum	+++	++++	++++	++++	++	±	—
2	Saline, 0.85 %	+++	+++	+++	+	—		
	Stored bovine serum	+++	+++	++	—	—		
	Fresh bovine serum	+++	+++	+++	++	—		

examined, served to increase the agglutination titre by one or two steps in the dilution series. In addition, it brought about an increase in the size of the rickettsial aggregates and rendered them more easily discernible. It might be noted in passing that, in some cases, the use of such a diluent also led to the disappearance of prozones (cf. Table 6).

The advantage of conglutinin in the micro-agglutination test for *C. burnetii* was readily apparent. Inactivated bovine serum was used in a dilution of 1:16 to 1:20 and mixed with guinea-pig serum diluted to contain two units of complement as determined by the technique of Hole and Coombs. (Two units generally corresponded to a 1:30 or 1:40 dilution.) Serial dilutions of the serum under test were made in this mixture of bovine and guinea-pig sera. Instead of guinea-pig complement, fresh bovine serum may be used with equal success.

TABLE 6. COMPARISON BETWEEN AUXI-AGGLUTINATION AND CONGLUTINATION EFFECTS IN MICROSCOPIC AGGLUTINATION TEST FOR *COXIELLA BURNETTI*

Q-fever-immune human serum		Agglutinating titre of serum					
Number	Diluent	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128
1	Saline, 0.85 %	++	+	±	—	—	—
	Bovine serum, 1 : 20	—	+++	+	±	—	—
	Bovine serum, 1 : 20 plus complement, 1 : 40	++++	+++	++	±	—	—
2	Saline, 0.85 %	+++	+++	+++	+	—	—
	Bovine serum, 1 : 20	++	+++	+++	++	—	—
	Bovine serum, 1 : 20 plus complement, 1 : 40	++	++	+++	+++	+++	±

Table 6 shows the results of two comparative tests, using three different diluents. Both auxi-agglutination (bovine serum without complement) and conglutination (bovine serum plus complement) are illustrated. It will be observed that conglutination gave results on the whole equal to or slightly better than those given by the auxi-agglutination method. Sera that are strongly anticomplementary, however, do not give any better results by the conglutination method than by the auxi-agglutination method. In general, the conglutination method affords some advantage over the simple microscopic agglutination method. However, it is more complicated to perform and requires freshly drawn bovine serum and complement, factors which frequently offset the advantages offered by the test. The practical utility of the conglutination method is more circumscribed than that of the microscopic agglutination test, which in itself is already highly sensitive.

Discussion

The microscopic agglutination test possesses a number of advantages over other agglutination methods. As the studies reported here show, it is more sensitive than either the macroscopic agglutination test or the capillary agglutination test, and the increased sensitivity is not obtained at a sacrifice of specificity. The test also possesses the advantages of being more economical than other techniques, the consumption of antigen being so small that the rickettsial suspension obtained from a single embryo provides adequate antigen to perform hundreds of tests.

In comparison with the complement fixation test, the microscopic agglutination test offers the advantage of greater sensitivity. If the sera to be tested are taken from patients during an infection or soon after their recovery, the sensitivity of the two reactions is not very different; but if a considerable interval elapses between the illness and taking the sera, the microscopic agglutination test is found to be patently superior to the complement fixation method in detecting antibodies to *C. burnetii*.

Another advantage of the microscopic agglutination test is that it can be performed with dried blood. This procedure is especially useful in large-scale epidemiological studies when it is difficult to obtain from each subject the amount of blood required to perform the usual macroscopic serological tests. In such cases, a drop of blood may be obtained by simple puncture and absorbed on a strip of filter paper. In the laboratory, the portion of paper containing the dried blood specimen is cut out and placed for half an hour in a tube containing a 1% formalin-saline solution, approximately three times the volume of the blood drop. The solution of extracted blood may be used directly in the microscopic agglutination test, and the method gives very good results with blood specimens dried for 60 or more days. The titre obtained by this procedure is only approximate, but more accurate quantitative methods can be used.

It was observed that the use of diluents containing high concentrations of salt (2.5-5%) in the microscopic agglutination test makes the reaction less clear-cut. The reaction is not perceptibly affected, however, by salt concentrations between 0.6% and 1.25%. In some respects, a concentration of 0.6% is preferable to 0.85%, as it avoids the formation of salt crystals ; this is helpful when agglutination tests are done with extracts of dried blood, since it makes the reaction easier to read.

For the usual purposes, such as diagnosis or epidemiological investigations, the use of " auxi-agglutination " or " conglutination " techniques does not appear to afford any special advantage over the basic microscopic agglutination method described here. These techniques may prove useful, however, in certain special cases where it is necessary to increase the sensitivity of the agglutination reaction. Because of the difficulty associated with having a readily accessible source of fresh bovine serum available, some experiments were undertaken on the use of various concentrations of bovine albumen (Armour). This material did not lend itself well to the purpose, however, because drying of the serum-antigen mixtures led to the formation of precipitates that rendered it difficult to read the agglutination. Similar difficulties were encountered when 0.07% gelatin was added to the physiological saline or phosphate buffers used as diluents.

RÉSUMÉ

L'auteur a mis au point une épreuve de micro-agglutination sur lame pour le sérodiagnostic de la fièvre Q. La sensibilité de ce test est dix fois plus grande que celle de l'agglutination en tube capillaire ou la macro-agglutination, et de beaucoup supérieure encore à celle du test de fixation du complément, surtout s'il s'agit de déceler des anticorps un certain temps après la fin de la maladie. Cette sensibilité peut être augmentée encore par l'addition de sérum bovin inactivé (auxo-agglutination), mais ce perfectionnement n'est pas nécessaire pour les épreuves courantes de diagnostic et les recherches épidémiologiques. Un autre avantage de ce test est de pouvoir être effectué avec du sang desséché sur papier filtre, ce qui le rend précieux dans les grandes enquêtes épidémiologiques.

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